



PCT/GB 2003 / 0 0 1 5 4 3



INVESTOR IN PEOPLE
REC'D 20 JUN 2003

WIPO

PCT

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 8 April 2003

THE PATENT OFFICE
B
- 5 APR 2002
NEWPORT

APR 02 E708918-3 D00239
P01/7700 0.00-0207900.2

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference PC/JM/P12115GB

2. Patent application number

(The Patent Office will fill in this part)

05 APR 2002

0207900.2

3. Full name, address and postcode of the or of each applicant (underline all surnames)

THE UNIVERSITY COURT OF THE UNIVERSITY
OF EDINBURGH
OLD COLLEGE
SOUTH BRIDGE
EDINBURGH EH8 9YL
UNITED KINGDOM

04001590001

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention "SCHIZOPHRENIA ASSOCIATED GENE (III)"

5. Name of your agent (if you have one)

CRUIKSHANK & FAIRWEATHER
19 ROYAL EXCHANGE SQUARE
GLASGOW G1 3AE
UNITED KINGDOM

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Patents ADP number (if you know it)

547002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

~~PERSONAL & CONFIDENTIAL~~
Continuation sheets of this form
~~S. 00P000-00-0 00P000~~

Description 30

Claim(s) -

Abstract -

Drawing(s) 9 + 9 *12*

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature *Cruijkshank & Fairweather*

Date *4/4/02*

CRUIKSHANK & FAIRWEATHER 4 APRIL 2002

12. Name and daytime telephone number of person to contact in the United Kingdom DR. P. CHAPMAN 0141-221-5767

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
- Write your answers in capital letters using black ink or you may type them.*
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.*
- Once you have filled in the form you must remember to sign and date it.*
- For details of the fee and ways to pay please contact the Patent Office.*

SCHIZOPHRENIA ASSOCIATED GENE (III)

The present invention relates to the identification of a gene which has been disrupted in a patient diagnosed as suffering from schizophrenia, as well as proteins encoded by the gene and antibodies thereto and to uses of such products as medicaments for treating schizophrenia and/or affective disorder. The invention also relates to methods for diagnosing patients suffering or predisposed to schizophrenia and/or affective disorder, as well as screens for developing novel treatment regimes for schizophrenia and/or affective disorder.

Schizophrenia and affective disorders such as Bipolar Affective Disorder are common and debilitating psychiatric disorders. Despite a wealth of information on the epidemiology, neuroanatomy and pharmacology of the illness, it is uncertain what molecular pathways are involved and how impairments in these affect brain development and neuronal function. Despite an estimated heritability of 60-80%, very little is known about the number or identity of genes involved in these psychoses. Although there has been recent progress in linkage and association studies, especially from genome-wide scans, these studies have yet to progress from the identification of susceptibility loci or candidate genes to the full characterisation of disease-causing genes (Berrettini, 2000).

The cloning of breakpoints in patients with chromosome abnormalities (translocations, inversions etc.) has proved instrumental in the identification of many disease genes

(e.g. Duchenne Muscular Dystrophy, Retinoblastoma, Wilm's Tumour, Familial Polyposis Coli, Fragile-X Syndrome, Polycystic Kidney Disease, many leukaemias and, very recently, a candidate speech and language disorder gene (Lai et al, 2001)). Such studies assume that the chromosomal breakpoints give rise to the clinical symptoms by either directly disrupting gene sequences or perturbing gene expression. In the latter case, it is considered that the breakpoint can still affect expression of a gene at a distance of at least up to 1Mb (Kleinjan & van Heyningen, 1998). In the same way that gene-trap mutagenesis can be used to identify disrupted mouse genes (Brennan & Skarnes, 1999), the physical "flag" created by a cytogenetic breakpoint provides a geographical pointer for the disease locus.

It is amongst the objects of the present invention to provide a gene and/or protein postulated to be involved with the development and/or symptoms associated with schizophrenia and/or affective disorder.

The present invention is based on the *PDE4B* gene and observations of the present inventors of an involvement of this gene and/or protein with schizophrenia and/or affective psychosis.

As will be seen the present invention is based on the molecular characterisation of a chromosomal rearrangement in a subject diagnosed as suffering from schizophrenia. A high-throughput Fluorescence *in situ* Hybridisation (FISH)-based approach has been adopted to map the chromosomal

breakpoints in these patients. Consultation of the sequence data at the breakpoint locus not only allows efficient FISH probe selections to be made by the targeting of coding regions, but proof of gene disruption can be made entirely by relating the exact position of probes to the genomic structure of a candidate gene. This removes the necessity for laborious physical mapping of breakpoint loci, a process that requires quantities of genomic DNA that are not necessarily available from some psychiatric patients or their derived lymphoblastoid cell lines.

The subject was diagnosed with schizophrenia and was the proband in a family where psychiatric illness was present in several members with chromosomal abnormalities.

As described herein, the FISH results reveal that the subject has a disruption in a brain expressed gene; namely the *PDE4B* gene, which is known to participate in molecular mechanisms responsible for modulating the strength of synaptic transmission.

In a first aspect the present invention provides use of a polynucleotide fragment comprising the *PDE4B* gene or a fragment, derivative or homologue thereof for the manufacture of a medicament for treating schizophrenia and/or affective disorder in a subject.

In another aspect the present invention provides use of a polypeptide fragment encoded by the *PDE4B* gene, or a fragment, derivative or homologue thereof for the manufacture of a medicament for treating schizophrenia and/or affect in a subject.

Schizophrenia and/or affective psychosis as used herein relates to schizophrenia, as well as other affective psychoses such as those listed in "The ICD-10 Classification of Mental and Behavioural Disorders" World Health Organization, Geneva 1992. Categories F20 to F29 inclusive includes Schizophrenia, schizotypal and delusional disorders. Categories F30 to F39 inclusive are Mood (affective) disorders that include bipolar affective disorder and depressive disorder. Mental Retardation is coded F70 to F79 inclusive. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). American Psychiatric Association, Washington DC. 1994. Include all conditions coded 295.xx (Schizophrenia and Other Psychotic Disorders) and 296.xx (Major Depressive Disorders and Bipolar Disorders). Mental Retardation is coded 315, 317, 318 and 319.

The *PDE4B* gene is located on chromosome 1 at cytogenetic position 1p31.2. The gene encodes a phosphodiesterase which shows homology to the dunce leaning and memory gene product of *Drosophila melanogaster*, Bolger et al, 1993. Two long (PDE4B1 and PDE4B3) and one short (PDE4B2) splice form are described herein. There is a core protein sequence of 525 amino acid residues shared by all three forms. On to this is added 39 N-terminal amino acid residues in the case of PDE4B2. Both of the long forms share an additional central stretch of 118 amino acid residues, but then diverge at the N-terminal end of the proteins; PDE4B1 has 93 specific residues and PDE4B3, 78.

It is predicted that only the PDE4B1 splice form (brain expressed) may be disrupted by the chromosomal abnormality observed in the patient and family.

Thus, references herein to the *PDE4B* gene are understood to relate to the sequences identified in Figures 1, 3 and 7 and references to the PDE4B protein sequence are understood to relate to the sequences identified in Figures 2, 4 and 6.

In certain jurisdictions claims to methods of treatment are permissible and so the skilled reader will appreciate that the *PDE4B* gene, or fragment, derivative or homologue thereof; or PDE4B protein, or functionally active fragment, derivative, or homologue thereof, may be administered to an individual as a method of treating an individual with schizophrenia and/or affective psychosis.

"Polynucleotide fragment" as used herein refers to a chain of nucleotides such as deoxyribose nucleic acid (DNA) and transcription products thereof, such as RNA. Naturally, the skilled addressee will appreciate the whole naturally occurring human genome is not included in the definition of polynucleotide fragment.

The polynucleotide fragment can be isolated in the sense that it is substantially free of biological material with which the whole genome is normally associated *in vivo*. The isolated polynucleotide fragment may be cloned to provide a recombinant molecule comprising the polynucleotide fragment. Thus, "polynucleotide fragment" includes double and single stranded DNA, RNA and

polynucleotide sequences derived therefrom, for example, subsequences of said fragment and which are of any desirable length. Where a nucleic acid is single stranded then both a given strand and a sequence or reverse complementary thereto is within the scope of the present invention.

In general, the term "expression product" or "gene product" refers to both transcription and translation products of said polynucleotide fragments. When the expression or gene product is a "polypeptide" (i.e. a chain or sequence of amino acids displaying a biological activity substantially similar (eg. 98%, 95%, 90%, 80%, 75% activity) to the biological activity of the protein), it does not refer to a specific length of the product as such. Thus, the skilled addressee will appreciate that "polypeptide" encompasses *inter alia* peptides, polypeptides and proteins. The polypeptide if required, can be modified *in vivo* and *in vitro*, for example by glycosylation, amidation, carboxylation, phosphorylation and/or post-translational cleavage.

The present invention further provides a recombinant or synthetic polypeptide for the manufacture of reagents for use as therapeutic agents in the treatment of schizophrenia and/or affective disorder. In particular, the invention provides pharmaceutical compositions comprising the recombinant or synthetic polypeptide together with a pharmaceutically acceptable carrier therefor.

The present invention further provides an isolated polynucleotide fragment capable of specifically hybridising to a related polynucleotide sequence from another species. In this manner, the present invention provides probes and/or primers for use in *ex vivo* and/or *in situ* detection and expression studies. Typical detection studies include polymerase chain reaction (PCR) studies, hybridisation studies, or sequencing studies. In principle any specific polynucleotide sequence fragment from the identified sequences may be used in detection and/or expression studies. The skilled addressee understands that a specific fragment is a fragment of the sequence which is of sufficient length, generally greater than 10, 12, 14, 16 or 20 nucleotides in length, to bind specifically to the sequence, under conditions of high stringency, as defined herein, and not bind to unrelated sequences, that is sequences from elsewhere in the genome of the organism other than an allelic form of the sequence or non-homologous sequences from other organisms.

"Capable of specifically hybridising" is taken to mean that said polynucleotide fragment preferably hybridises to a related or similar polynucleotide sequence in preference to unrelated or dissimilar polynucleotide sequences.

The invention includes polynucleotide sequence(s) which are capable of specifically hybridising to an polynucleotide fragment as described herein or to a part thereof without necessarily being completely complementary or reverse complementary to said related polynucleotide

sequence or fragment thereof. For example, there may be at least 50%, or at least 75%, at least 90%, or at least 95% complementarity. Of course, in some cases the sequences may be exactly reverse complementary (100% reverse complementary) or nearly so (e.g. there may be less than 10, typically less than 5 mismatches). Thus, the present invention also provides anti-sense or complementary nucleotide sequence(s) which is/are capable of specifically hybridising to the disclosed polynucleotide sequence. If a specific polynucleotide is to be used as a primer in PCR and/or sequencing studies, the polynucleotide must be capable of hybridising to related nucleic acid and capable of initiating chain extension from 3' end of the polynucleotide, but not able to correctly initiate chain extension from unrelated sequences.

If a polynucleotide sequence of the present invention is to be used in hybridisation studies to obtain or identify a related sequence from another organism the polynucleotide sequence should preferably remain hybridised to a sample polynucleotide under stringent conditions. If desired, either the test or sample polynucleotide may be immobilised. Generally the test polynucleotide sequence is at least 10, 14, 20 or at least 50 bases in length. It may be labelled by suitable techniques known in the art. Preferably the test polynucleotide sequence is at least 200 bases in length and may even be several kilobases in length. Thus, either a denatured sample or test sequence can be first bound to a support. Hybridization can be

effected at a temperature of between 50 and 70°C in double strength SSC (2xNaCl 17.5g/l and sodium citrate (SC) at 8.8g/l) buffered saline containing 0.1% sodium dodecyl sulphate (SDS). This can be followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC concentration. Depending upon the degree of stringency required, and thus the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1%SDS, half strength SSC containing 0.1%SDS and one tenth strength SSC containing 0.1%SDS. Sequences having the greatest degree of similarity are those the hybridisation of which is least affected by washing in buffers of reduced concentration. It is most preferred that the sample and inventive sequences are so similar that the hybridisation between them is substantially unaffected by washing or incubation in standard sodium citrate (0.1 x SSC) buffer containing 0.1%SDS.

Oligonucleotides may be designed to specifically hybridise to *PDE4B* nucleic acid. They may be synthesised, by known techniques and used as primers in PCR or sequencing reactions or as probes in hybridisations designed to detect the presence of a mutated or normal *PDE4B* gene in a sample. The oligonucleotides may be labelled by suitable labels known in the art, such as, radioactive labels, chemiluminescent labels or fluorescent labels and the like.

The term "oligonucleotide" is not meant to indicate any particular length of sequence and encompasses nucleotides of preferably at least 10b (e.g. 10b to 1kb) in length, more preferably 12b-500b in length and most preferably 15b to 100b.

The oligonucleotides may be designed with respect to any of the sequences described herein and may be manufactured according to known techniques. They may have substantial sequence identity (e.g. at least 50%, at least 75%, at least 90% or at least 95% sequence identity) with one of the strands shown therein or an RNA equivalent, or with a part of such a strand. Preferably such a part is at least 10, at least 30, at least 50 or at least 200 bases long. It may be an open reading frame (ORF) or a part thereof.

Oligonucleotides which are generally greater than 30 bases in length should preferably remain hybridised to a sample polynucleotide under one or more of the stringent conditions mentioned above. Oligonucleotides which are generally less than 30 bases in length should also preferably remain hybridised to a sample polynucleotide but under different conditions of high stringency. Typically the melting temperature of an oligonucleotide less than 30 bases may be calculated according to the formula of; 2°C for every A or T, plus 4°C for every G or C, minus 5°C . Hybridization may take place at or around the calculated melting temperature for any particular oligonucleotide, in 6 x SSC and 1% SDS. Non specifically hybridised

oligonucleotides may then be removed by stringent washing, for example in 3 x SSC and 0.1% SDS at the same temperature. Only substantially similar matched sequences remain hybridised i.e. said oligonucleotide and corresponding test nucleic acid.

When oligonucleotides of generally less than 30 bases in length are used in sequencing and/or PCR studies, the melting temperature may be calculated in the same manner as described above. The oligonucleotide may then be allowed to anneal or hybridise at a temperature around the oligonucleotides calculated melting temperature. In the case of PCR studies the annealing temperature should be around the lower of the calculated melting temperatures for the two priming oligonucleotides. It is to be appreciated that the conditions and melting temperature calculations are provided by way of example only and are not intended to be limiting. It is possible through the experience of the experimenter to vary the conditions of hybridisation and thus anneal/hybridise oligonucleotides at temperatures above their calculated melting temperature. Indeed this can be desirable in preventing so-called non-specific hybridisation from occurring.

It is possible when conducting PCR studies to predict an expected size or sizes of PCR product(s) obtainable using an appropriate combination of two or more oligonucleotides, based on where they would hybridise to the sequences described herein. If, on conducting such a PCR on a sample of DNA, a fragment of the predicted size is

obtained, then this is predictive that the DNA encodes a homologous sequence from a test organism.

Proteins for all the applications described herein can be produced by cloning the gene for example into plasmid vectors that allow high expression in a system of choice e.g. insect cell culture, yeast, animal cells, bacteria such as *Escherichia coli*. To enable effective purification of the protein, a vector may be used that incorporates an epitope tag (or other "sticky" extension such as His6) onto the protein on synthesis. A number of such vectors and purification systems are commercially available.

The polynucleotide fragment can be molecularly cloned into a prokaryotic or eukaryotic expression vector using standard techniques and administered to a host. The expression vector is taken up by cells and the polynucleotide fragment of interest expressed, producing protein.

It will be understood that for the particular polypeptides embraced herein, natural variations such as may occur due to polymorphisms, can exist between individuals or between members of the family. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. All such derivatives showing the recognised activity are included within the scope of the invention. For example, for the purpose of the present invention conservative replacements may be made

between amino acids within the following groups:

- (I) Alanine, serine, threonine;
- (II) Glutamic acid and aspartic acid;
- (III) Arginine and leucine;
- (IV) Asparagine and glutamine;
- (V) Isoleucine, leucine and valine;
- (VI) Phenylalanine, tyrosine and tryptophan

Moreover, recombinant DNA technology may be used to prepare nucleic acid sequences encoding the various derivatives outlined above.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in a different codon which is still capable of coding for the same amino acid, e.g. the codon for amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of polypeptides from nucleotide sequences described herein or fragments thereof, use can be made of a derivative nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequences shown in the Figures.

The polynucleotide fragments of the present invention are preferably linked to regulatory control sequences. Such control sequences may comprise promoters, operators, inducers, enhancers, silencers, ribosome binding sites, terminators etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art.

A polynucleotide fragment according to the present invention can be ligated to various expression controlling sequences, resulting in a so called recombinant nucleic acid molecule. Thus, the present invention also includes an expression vector containing an expressible nucleic acid molecule. The recombinant nucleic acid molecule can then be used for the transformation of a suitable host.

Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art (e.g. Rodriguez, R.L. and Denhardt, D.T., Edit., Vectors: a survey of molecular cloning vectors and their uses, Butterworths, 1988, or Jones et al., Vectors: Cloning Applications: Essential Techniques (Essential techniques series), John Wiley & Son. 1998).

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Sambrook, et al. (Molecular Cloning: a laboratory manual Cold Spring Harbour Laboratory, 1989).

The present invention also relates to a transformed cell containing the polynucleotide fragment in an expressible form. "Transformation", as used herein, refers to the introduction of a heterologous polynucleotide fragment into a host cell. The method used may be any known in the art, for example, direct uptake, transfection transduction or electroporation (Current Protocols in Molecular Biology, 1995. John Wiley and Sons Inc.). The

heterologous polynucleotide fragment may be maintained through autonomous replication or alternatively, may be integrated into the host genome. The recombinant nucleic acid molecules preferably are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted polynucleotide fragment, e.g. tetracycline responsive promoter, thymidine kinase promoter, SV-40 promoter and the like.

Suitable hosts for the expression of recombinant nucleic acid molecules may be prokaryotic or eukaryotic in origin. Hosts suitable for the expression of recombinant nucleic acid molecules may be selected from bacteria, yeast, insect cells and mammalian cells.

In another aspect the present invention also relates to a method of diagnosing schizophrenia and/or affective psychosis disease or susceptibility to schizophrenia and/or affective psychosis in an individual, wherein the method comprises determining if the *PDE4B* gene in the individual has been disrupted by a mutation or chromosomal rearrangement.

The methods which may be employed to elucidate such a mutation or chromosomal rearrangement are well known to those of skill in the art and could be detected for example using PCR or in hybridisation studies using suitable probes which could be designed to span an identified mutation site or chromosomal breakpoint in the *PDE4B* gene, such as the breakpoint identified by the present inventors and described herein.

Once a particular polymorphism or mutation has been identified it may be possible to determine a particular course of treatment. For example it is known that some forms of treatment work for some patients, but not all. This may in fact be due to mutations in the *PDE4B* gene or surrounding sequence, it may therefore be possible to determine a treatment strategy using current therapies, based on a patient's genotype.

It will be appreciated that mutations in the gene sequence or controlling elements of a gene, eg. a promoter and/or enhance can have subtle effects such as affecting mRNA splicing/stability/activity and/or control of gene expression levels, which can also be determined.

Also the relative levels of RNA can be determined using for example hybridisation or quantitative PCR as a means to determine if the *PDE4B* gene has been disrupted.

Moreover the presence and/or levels of the *PDE4B* gene products themselves can be assayed by immunological techniques such as radioimmunoassay, Western blotting and ELISA using specific antibodies raised against the gene products. The present invention also therefore relates to antibodies specific for a *PDE4B* gene product and uses thereof in diagnosis and/or therapy.

A further aspect of the present invention therefore provides antibodies specific to the polypeptides of the present invention or epitopes thereof. Production and purification of antibodies specific to an antigen is a matter of ordinary skill, and the methods to be used are

clear to those skilled in the art. The term antibodies can include, but is not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanised or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope binding fragments of any of the above. Such antibodies may be used in modulating the expression or activity of the particular polypeptide, or in detecting said polypeptide *in vivo* or *in vitro*.

Using the sequences disclosed herein, it is possible to identify related sequences in other animals, such as mammals, with the intention of providing an animal model for psychiatric disorders associated with the improper functioning of the nucleotide sequences and proteins of the present invention. Once identified, the homologous sequences can be manipulated in several ways common to the skilled person in order to alter the functionality of the nucleotide sequences and proteins homologous to those of the present invention. For example, "knock-out" animals may be created, that is, the expression of the genes comprising the nucleotide sequences homologous to those of the present invention may be reduced or substantially eliminated in order to determine the effects of reducing or substantially eliminating the expression of such genes. Alternatively, animals may be created where the expression of the nucleotide sequences and proteins homologous to those of the present invention are upregulated, that is,

the expression of the genes comprising the nucleotide sequences homologous to those of the present invention may be increased in order to determine the effects of increasing the expression of these genes. In addition to these manipulations, substitutions, deletions and additions may be made to the nucleotide sequences encoding the proteins homologous to those of the present invention in order to effect changes in the activity of the proteins to help elucidate the function of domains, amino acids, etc. in the proteins. Furthermore, the sequences of the present invention may also be used to transform animals to the manner described above. The manipulations described above may also be used to create an animal model of schizophrenia and/or affective psychosis associated with the improper functioning of the nucleotide sequences and/or proteins of the present invention in order to evaluate potential agents which may be effective for combatting psychotic disorders, such as schizophrenia and affective psychosis.

Thus, the present invention also provides for screens for identifying agents suitable for preventing and/or treating, schizophrenia and/or affective disorder associated with disruption or alteration in the expression of the *PDE4B* gene and/or its gene products. Such screens may easily be adapted to be used for the high throughput screening of libraries of compounds such as synthetic, natural or combinatorial compound libraries.

Thus, the *PDE4B* gene products according to the present invention can be used for the *in vivo* or *in vitro* identification of novel ligands or analogs thereof. For this purpose binding studies can be performed with cells transformed with nucleotide fragments according to the invention or an expression vector comprising a polynucleotide fragment according to the invention, said cells expressing the *PDE4B* gene products according to the invention.

Alternatively also the *PDE4B* gene products according to the invention as well as ligand-binding domains thereof can be used in an assay for the identification of functional ligands or analogs for the *PDE4B* gene products.

Methods to determine binding to expressed gene products as well as *in vitro* and *in vivo* assays to determine biological activity of gene products are well known. In general, expressed gene product is contacted with the compound to be tested and binding, stimulation or inhibition of a functional response is measured.

Thus, the present invention provides for a method for identifying ligands for *PDE4B* gene products, said method comprising the steps of:

- a) introducing into a suitable host cell a polynucleotide fragment according to the invention;
- b) culturing cells under conditions to allow expression of the polynucleotide fragment;
- c) optionally isolating the expression product;

d) bringing the expression product (or the host cell from step b)) into contact with potential ligands which will possibly bind to the protein encoded by said polynucleotide fragment from step a);

e) establishing whether a ligand has bound to the expressed protein; and

f) optionally isolating and identifying the ligand.

As a preferred way of detecting the binding of the ligand to the expressed protein, also signal transduction capacity may be measured.

Compounds which activate or inhibit the function of *PDE4B* gene products may be employed in therapeutic treatments to activate or inhibit the polypeptides of the present invention.

The present invention will now be further described by way of Example and with reference to the Figures which show:

Figure 1 shows the *PDE4B1* nucleic acid sequence;

Figure 2 shows the *PDE4B1* protein sequence;

Figure 3 shows the *PDE4B3* nucleic acid sequence;

Figure 4 shows the *PDE4B3* protein sequence;

Figure 5 shows the *PDE4B2* nucleic acid sequence;

Figure 6 shows the *PDE4B2* protein sequence;

Figure 7 a) Ideogram representation of balanced translocation between chromosomes 1 and 16 in the patient.

Figure 8 Genomic arrangements of the *PDE4B* gene disrupted in the subject. The two long transcripts of the *PDE4B* gene are shown. FISH showed the breakpoint was

within a gap in the genome sequence between BACs RPCI-11 433N2 and RPCI-11 442I1. This positioned the breakpoint between the first and second exons of the *PDE4B1* form of the gene (acc. L20966). A long-range PCR product FISH probe corresponding to the genomic region encompassing the 1a exons of *PDE4B1* confirmed that the gene was disrupted between exon pairs 1a and exon 2 (i.e. only *PDE4B1* transcripts are directly disrupted by the chromosome abnormality).

Materials and Methods

Psychiatric evaluation

The subject (male) is the proband in a family segregating a t(1;16) balanced reciprocal translocation. He gave full informed consent to the study. His diagnosis of chronic schizophrenia was confirmed by SADS-L structured interview and a consensus reached by two psychiatrists (WM and DB). He does not have mental retardation. Other members of his near family also gave consent to participate in this study, none of whom had current mental illness (several are below the age of risk for psychiatric illness). There was also a history of mental illness (major depressive disorder) in members of the extended family who were known to be translocation carriers, but they could not be approached for confirmation at the time of the current study (not detailed in Figure 7b). An unrelated individual (now deceased) with DSM-IV chronic

schizophrenia without learning disability also had a t(1;16) balanced translocation with the same breakpoints (at the resolution of G-banding).

Lymphocyte extraction and metaphase chromosome preparation

Lymphocytes were extracted from 7mls of patient blood (for storage and generation of EBV-transformed cell lines) using density gradient separation (Histopaque-1077, Sigma). In order to generate metaphase-arrested chromosomes for cytogenetic analysis, 0.8mls of patient blood were cultured for 71hrs in medium containing phytohaemagglutinin (Peripheral Blood Medium, Sigma). The short-term cultures were treated with colcemid for one hour followed by a conventional fixing procedure. Fixed chromosomes were dropped onto microscope slides and stored for 1 week prior to use in FISH experiments.

Selection of YAC clones for FISH probe synthesis

YAC clones were selected from the Whitehead/MIT map of the relevant chromosome in the cytogenetic intervals within which the breakpoints were adjudged to lie. YACs were obtained from the HGMP Resource Centre, Babraham Bioincubator, Babraham, Cambridge, UK (<http://www.hgmp.mrc.ac.uk/>). Clone DNA was prepared by standard methods and PCR amplified using primers designed against consensus sequence elements within the archetypal Alu repeat (Breen et al, 1992). This "Alu-PCR" gives a representative spread of non-repetitive sequence over the

full length of the YAC and generates a better FISH probe than native YAC DNA. Alu-PCR was performed using the Expand Long Template PCR kit (Roche). Cycling conditions: 94°C - 45s, 55°C - 30s, 68°C - 8min: 35 cycles. 68°C - 10min final extension.

Fluorescence in situ hybridisation (FISH) protocol

Probe template DNA (pooled Alu-PCR products, BAC clone DNA, cosmid clone DNA or long-range PCR products) was labelled by nick translation and hybridised to patient metaphase spreads using standard FISH methods. Slides were counterstained with DAPI in Vectashield anti-fade solution (Vector laboratories). The Zeiss Axioskop fluorescence microscope with a chroma number 81000 multi-spectral filter set. Images were captured using Vysis SmartCapture extension running within IP Lab spectrum. FISH signals observed on derived chromosomes dictated the selection of further clones required to "walk" towards the breakpoint. Breakpoint-spanning FISH probes have signals on a normal chromosome and on both derived chromosomes.

Resolution of breakpoint position

BAC clones corresponding to positive YAC regions were arranged into contigs by consulting the Washington University FPC

(<http://www.genome.wustl.edu/gsc/human/Mapping/index.shtml>),
UCSC GoldenPath Draft Human Genome Browser
(<http://genome.ucsc.edu/goldenPath/hgTracks.html>) and

Ensembl (<http://www.ensembl.org/>) databases. BAC clones were supplied by BACPAC Resources, Oakland, California, USA (<http://www.chori.org/bacpac/>). Clone selection was biased to gene-containing BACs. Once a breakpoint-spanning BAC was identified, the position of the breakpoint in relation to candidate gene exons was determined by FISH probes generated from chromosome-specific library cosmids (HGMP Resource centre) or precisely positioned, repeat element-free long-range PCR products (Expand long range PCR kit, Roche; see below for primer sequences). Cycling conditions: 94°C - 45s, 52°C - 30s, 68°C - 11min: 35 cycles. 68°C - 15min final extension. Cosmids were isolated by probing the appropriate chromosome-specific library filters (HGMP-RC) with isotopically labelled exon-specific PCR products.

PCR primers

Long-range PCR for FISH probe templates:

PDE4B3a; GTCAGACAAATCCAAATGGAGAG, PDE4B3b;
CTTCTCCTGTCACTTTCCTTCA.

Cycling conditions: 94°C - 2min initial denaturation. 94°C - 1min, 52°C - 1min, 72°C - 75s: 33 cycles. 72°C - 15min final extension.

The balanced translocation, t(1;16)(p31.2;q21), in this family results in two breakpoints (Fig.1b). Genomic sequence at 16q21 is not complete. The only known gene in the vicinity of the breakpoint region is Cadherin 8 (*CDH8*, acc. AB035305). However, FISH experiments using BACs

containing this gene indicate that it is not disrupted (data not shown).

In contrast, on chromosome 1p31.2 FISH identified two non-overlapping BAC clones (RP11-433N2, acc. AL513493 and RP11-442I1, acc. AL391359) which reside on either side of the breakpoint in this patient. The breakpoint-containing genomic region between these two BAC clones has yet to be sequenced (see Figure 8). Database annotation of the two BAC clones together with BLAST mapping of exons onto genomic sequence indicated that this locus contains a cAMP phosphodiesterase gene, *PDE4B*. Two cDNAs corresponding to longer transcript forms of this gene (denoted *PDE4B1*, acc. L20966 and *PDE4B3*, acc. U85048, respectively) have been previously characterised (Bolger et al, 1994; Huston et al, 1997). Long-range PCR product FISH (Figure 8) confirmed that the *PDE4B1* transcript is directly disrupted by the breakpoint (although additional position-effect perturbation of *PDE4B3* expression cannot be ruled out). Huston et al. (1997) have previously shown that the *PDE4B1* transcript encodes an alternative N-terminal peptide sequence. In addition, they demonstrated that only this form is expressed in the brain. It is therefore predicted that this patient will have a reduction in the levels of functional *PDE4B* in the brain.

Discussion

The present inventors have identified a subject with DSMIV chronic schizophrenia in whom chromosome translocation events have disrupted brain-expressed genes that are also functional disease candidates. Without wishing to be bound by theory it is hypothesised that the disruption of the PDE4B gene by a chromosomal breakpoint (and the resulting reduced gene dosage) is the principal underlying cause of psychiatric disease in this patient.

The gene disrupted in this patient is both expressed in the brain and participates in key physiological processes in the CNS. Notably, the gene may be involved in the alteration of the strength of synaptic/neural transmission, a phenomenon known as long-term potentiation (LTP). LTP is postulated to underlie cognitive functions such as learning and memory. Moreover, cognitive testing has previously established that these functions are frequently affected in patients with schizophrenia.

PDE4B

Stimulation of the G protein coupled receptor/heterotrimeric G protein pathway results in the synthesis of the secondary messenger, cAMP, by members of the adenylyl cyclase family of enzymes. This secondary messenger triggers a well-characterised signalling cascade that is principally mediated by cAMP-dependent protein kinase A (PKA) and cAMP-responsive transcription factor, CREB, both of which have been implicated in the molecular

pathways of LTP (Abel & Latal, 2001). cAMP signalling is attenuated by its breakdown by members of the phosphodiesterase enzyme family. Four members of the *PDE4* sub-family of cAMP phosphodiesterases have been identified to date (*PDE4A-PDE4D*). These four genes are the human homologues of the *Drosophila* learning and memory mutant gene, *Dunce*. The long form of the *PDE4B* protein, *PDE4B1*, is the only splice form with brain expression and the present inventors have shown that it is disrupted in the subject. Anti-*PDE4B* antibodies revealed expression within the inferior olive, the hypothalamus, the ventral striatum, the cerebellar molecular layer, globus pallidus, nucleus accumbens and substantia nigra (Cherry & Davis, 1999). The authors of this expression study suggested that *PDE4B* expression strongly correlates with brain areas underlying reward and affect in mammals. In addition, *PDE4* proteins are recognised as the molecular targets for Rolipram, a drug with anti-depressant effects. Rolipram inhibition of *PDE4* activity has been shown to improve long-term hippocampal LTP and spatial memory in mice (Barad et al, 1998 and Bach et al, 1991). The (heterozygous) disruption to *PDE4B1* described here may be equivalent to 50% reduction of protein product in the brain. This could result in a greater cAMP half-life and a concomitant increase in the activation of downstream cAMP targets.

In addition, the disruption to *PDE4B* shows reduced penetrance as not all translocation carriers present with psychiatric illness (although all members of the extended family with psychiatric illness possess the translocation karyotype; data not shown).

REFERENCES

1. Berrettini W.H. (2000) Genetics of psychiatric disease. *Annu. Rev. Med.* 51:465 - 79.
2. Lai C.S, Fisher S.E, Hurst J.A, Vargha-Khadem F, Monaco A.P. (2001) A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature.* 413(6855):519 - 23.
3. Kleinjan D.J, van Heyningen V. (1998) Position effect in human genetic disease. *Hum Mol Genet.* 7(10):1611 - 8.
4. Brennan J, Skarnes W.C. (1999) Gene trapping in mouse embryonic stem cells. *Methods Mol. Biol.* 97:123 - 38.
5. Breen M, Arveiler B, Murray I, Gosden J.R, Porteous D.J. (1992) YAC mapping by FISH using Alu-PCR-generated probes. *Genomics.* 13(3):726 - 30.
6. Bolger G.B, Rodgers L, Riggs M. (1994) Differential CNS expression of alternative mRNA isoforms of the mammalian genes encoding cAMP-specific phosphodiesterases. *Gene.* 149(2):237 - 44.
7. Huston E, Lumb S, Russell A, Catterall C, Ross A.H, Steele M.R, Bolger G.B, Perry M.J, Owens R.J, Houslay M.D. (1997) Molecular cloning and transient expression in COS7 cells of a novel human PDE4B cAMP-specific phosphodiesterase, HSPDE4B3. *Biochem J.* 328 (Pt 2):549 - 58.
8. Abel T, Lattal K.M. (2001) Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol.* 11(2):180 - 7.

9. Cherry J.A, Davis R.L. (1999) Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J Comp Neurol.* 407(2):287 - 301.
10. Barad M, Bourtchouladze R, Winder D.G, Golan H, Kandel E. (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci U S A.* 95(25):15020 - 5.
11. Bach M.E, Barad M, Son H, Zhuo M, Lu Y.F, Shih R, Mansuy I, Hawkins R.D, Kandel E.R. (1999) Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A.* 96(9):5280 - 5.

Figure 1

PDE4B1 (acc. L20966) Nucleic acid sequence

```
1  gcgccgcg cgggtgcagca gaggcgcctc gggcaggagg agggcggtt ctgcgagggc
61  agcctgaggt attaaaaagt gtcagcaaac tgcattgaat aacagacatc ctaagagggg
121 atattttcca cctctataat gaagaaaagc aggagtgtga tgacggtgat ggctgatgat
181 aatgttaaag attattttga atgtagcttg agtaaactct acagttcttc cagtaacaca
241 cttgggatcg acctctggag agggagaagg tgttgctcag gaaacttaca gttaccacca
301 ctgtctcaaa gacagagtga aagggaagg actcctgagg gagatggtat ttccaggccg
361 accacactgc ctttgacaac gcttccaagc attgctatta caactgtaag ccaggagtgc
421 tttgatgtgg aaaatggccc ttccccaggt cggagtccac tggatcccca ggccagctct
481 tccgctgggc tggactttca cgccaccttt cctgggcaca gccagcgag agagtcatct
541 ctctacagat cagacagcga ctatgacttg tcaccaaagg cgatgtcgag aaactcttct
601 cttccaagcg agcaacacgg cgatgacttg attgtaactc cttttgcccc ggtccttgcc
661 agcttgcgaa gtgtgagaaa caacttcaat atactgacaa accttcattg tacatctaac
721 aagaggtccc cagctgctag tcagctcctt gtctccagag tcaaccaca agaagaatct
781 tatcaaaaat tagcaatgga aacgctggag gaattagact ggtgtttaga ccagctagag
841 accatacaga cctaccggtc tgtcagttag atggcttcta acaagttcaa aagaatgctg
901 aaccgggagc tgacacacct ctcagagatg agccgatcag ggaaccagggt gtctgaatac
961 atttcaataa ctttcttaga caagcagaat gatgtggaga tcccatctcc taccagaaa
1021 gacagggaga aaaagaaaaa gcagcagctc atgaccaga taagtggagt gaagaaatta
1081 atgcatagtt caagcctaaa caatacaagc atctcacgct ttggagtcaa cactgaaaat
1141 gaagatcacc tggccaagga gctggaagac ctgaacaaat ggggtcttaa catctttaat
1201 gtggctggat attctcaca tagaccctta acatgcatca tgtatgctat attccaggaa
1261 agagacctcc taaagacatt cagaatctca tctgacacat ttataaccta catgatgact
1321 ttagaagacc attaccattc tgacgtggca tatcacaaca gcctgcacgc tgctgatgta
1381 gccagtcga cccatgttct ctttcttaca ccagcattag acgtgtctt cacagatttg
1441 gagatcctgg ctgccatttt tgcagctgcc atccatgacg ttgatcatcc tggagtctcc
1501 aatcagtttc tcatcaacac aaattcagaa cttgctttga tgtataatga tgaatctgtg
1561 ttggaaaatc atcaccttgc tgtgggtttc aaactgctgc aagaagaaca ctgtgacatc
1621 ttcattgaatc tcaccaagaa acactcagga agatggttat tgacatgggtg
1681 ttagcaactg atatgtctaa acatatgagc ctgctggcag acctgaagac aatggtagaa
1741 aagaaagaa ttacaagttc aggcgttctt ctctagaca actataccga tgcattcag
1801 gtccttcgca acatggtaca ctgtgcagac ctgagcaacc ccaccaagtc cttggaattg
1861 tatcggcaat ggacagaccg catcatggag gaatttttcc agcagggaga caaagagcgg
1921 gagaggggaa tggaaattag ccaatgtgtt ccattgtggg agacatgggc agatttggtg
1981 cagctgatg ctcaggacat tctcgatacc ttagaagata acaggaactg gtatcagagc
2041 atgatactc aaagtccctc accaccactg gacgagcaga acagggactg ccagggtctg
2101 atggagaagt ttcagtttga actgactctc gatgaggaag attctgaagg acctgagaag
2161 gagggagagg gacacagcta tttcagcagc acaaagacgc tttgtgtgat tgatccagaa
2221 aacagagatt ccctgggaga gactgacata gacattgcaa cagaagacaa gtccccctg
2281 gatacataat cccctctcc ctgtggagat gaacattcta tccttgatga gcatgccagc
2341 tatgtggtag ggcagccca ccatgggggc caagacctgc acaggacaag ggccacctgg
2401 cctttcagtt acttgagttt ggagtcagaa agcaagacca ggaagcaaat agcagctcag
2461 gaaatccac ggttgacttg ccttgatggc aagcttgggt gagagggctg aagctgttgc
2521 tgggggccga ttctgatcaa gacacatggc ttgaaaatgg aagacacaaa actgagagat
2581 cattctgcac taagtttcgg gaacttatcc ccgacagtga ctgaactcac tgactaataa
2641 cttcatttat gaatcttctc acttgcctct ttgtctgcca acctgtgtgc ctttttgta
2701 aaacattttc atgtctttta aatgcctgtt gaatacctgg agtttagtat caacttctac
2761 acagataagc tttcaaagtt gacaaacttt tttgactctt tctggaaaag ggaaagaaaa
2821 tagtcttctc tctttcttgg gcaatatcct tcactttact acagttactt ttgcaaacag
2881 acagaaagga tacacttcta accacatttt acttccttcc cctgttgtcc agtccaactc
2941 cacagtcact cttaaaactt ctctctgttt gcctgcctcc aacagtactt ttaactttt
3001 gctgtaaaca gaataaaatt gaacaaatta ggggtagaa aggagcagtg gtgtcgttca
3061 ccgtgagagt ctgcatagaa ctcagcagtg tgccctgctg tgtcttgac cctgcaatgc
3121 ggcgcg
```

Figure 2

PDE4B1 Protein sequence

MPKERSVMTAMADDNYKDYFECSHSKSG358SNLEGLWRERRGCSGNLQLEPNSQ
ROSERARITPGDGLSRPFTLFTTPTSTALEVSGECEDVENGPSPGRSPLDPOASS
SAGLVLHATFPCHSORRESELYRSPSDYDLSPKAMSRNSSLPSEOHGDDLIVTFPAQ
VLASERSVRNNTTILTNLHGTSNKRSPAASOPPVSRRVNPQLESGHANN
LPSDLOKRAHFKQOQIMQSGAKDSSSNITPSRQVNTRENDILAK
LNNHGLHNNACSHNRLNQNVAHNCEDLKKRNHSEDMITAMMNLAD
LDVATINSLIADKASSTVLEHCPADAWLLEETLAAZTAYASTDUN
LNNHGLHNNACSHNRLNQNVAHNCEDLKKRNHSEDMITAMMNLAD
LPSDLOKRAHFKQOQIMQSGAKDSSSNITPSRQVNTRENDILAK
LNNHGLHNNACSHNRLNQNVAHNCEDLKKRNHSEDMITAMMNLAD
LPSDLOKRAHFKQOQIMQSGAKDSSSNITPSRQVNTRENDILAK
LNNHGLHNNACSHNRLNQNVAHNCEDLKKRNHSEDMITAMMNLAD

Figure 3

PDE4B3 (acc. U85048) Nucleic acid sequence

```
1  atgacagcaa aagattcttc aaaggaactt actgcttctg aacctgaggt ttgcataaag
61  acttttcaagg agcaaattgca tttagaactt gagcttccga gattaccagg aaacagacct
121 acatctccta aaattttctcc acgcagttca ccaaggaact caccatgctt tttcagaaag
181 ttactgggtga ataaaagcat tcggcagcgt cgtcgcttca ctgtggctca tacatgcttt
241 gatgtggaaa atggcccttc cccagggtcgg agtccactgg atccccaggc cagctcttcc
301 gctgggctgg tacttcacgc cacccttctc gggcacagcc agcgagaga gtcattttctc
361 tacagatcag acagcgacta tgactttgtca ccaaaggcga tgtcgagaaa ctcttctctt
421 ccaagcgagc aacacggcga tgacttgatt gtaactcctt ttgcccaggc ccttgccagc
481 ttgcgaagtg tgagaaacaa cttcactata ctgacaaacc ttcatggtac atctaacaag
541 aggtccccag ctgctagtca gcctcctgtc tccagagtca acccacaaga agaattcttat
601 caaaaattag caatggaaac gctggaggaa ttagactggg gtttagacca gctagagacc
661 atacagacct accggtctgt cagttagatg gcttctaaca agttcaaaag aatgctgaac
721 cgggagctga cacacctctc agagatgagc cgatcaggga accagggtgc tgaatacatt
781 tcaaatactt tcttagacaa gcagaatgat gtggagatcc catctcctac ccagaaagac
841 agggagaaaa agaaaaagca gcagctcatg acccagataa gtggagtga gaaattaatg
901 catagttcaa gcctaaacaa tacaagcatc tcacgctttg gagtcaacac tgaatatgaa
961 gatcacctgg ccaaggagct ggaagacctg aacaaatggg gtcttaacat ctttaatgtg
1021 gctggatatt ctcaaatag acccctaaca tgcattcatgt atgctatatt ccaggaaaga
1081 gacctcctaa agacattcag aatctcatct gacacattta taacctacat gatgacttta
1141 gaagaccatt accattctga cgtggcatac cacaacagcc tgcacgctgc tgatgtagcc
1201 cagtcgaccc atgttctcct ttctacacca gcattagacg ctgtcttcac agatttggag
1261 atcctggctg ccatttttgc agctgccatc catgacgttg atcatcctgg agtctccaat
1321 cagtttctca tcaacacaaa ttcagaactt gctttgatgt ataatgatga atctgtgttg
1381 gaaaatcatc accttgctgt gggtttcaaa ctgctgcaag aagaacactg tgacatcttc
1441 atgaatctca ccaagaagca gcgtcagaca ctcaggaaga tggttattga catgggtgta
1501 gcaactgata tgtctaaaca tatgagcctg ctggcagacc tgaagacaat ggtagaaacg
1561 aagaaagtta caagttcagg cgttcttctc ctagacaact ataccgatcg cattcaggtc
1621 cttcgcaaca tggtagactg tgcagacctg agcaacccca ccaagtcctt ggaattgtat
1681 cggcaatgga cagaccgat catggaggaa tttttccagc agggagaca agagcgggag
1741 aggggaatgg aaattagccc aatgtgtgat aaacacacag cttctgtgga aaaatcccag
1801 gttgggttca tcgactacat tgtccatcca ttgtgggaga catgggcaga tttggtacag
1861 cctgatgctc aggacattct cgatacctta gaagataaca ggaactggta tcagagcatg
1921 atacctcaaa gtccctcacc accactggac gagcagaaca gggactgcca gggctctgatg
1981 gagaagtttc agtttgaact gactctcgat gaggaagatt ctgaaggacc tgagaaggag
2041 ggagagggac acagctatct cagcagcaca aagacgcttt gtgtgattga tccagaaaac
2101 agagattccc tgggagagac tgacatagac attgcaacag aagacaagtc ccccgtagat
2161 aca
```

Figure 4

PDE4B3 Protein sequence

V I A R D S S K L E T T A S T F E V G L I T K E O M H I T T A T L E R I P E N R E I S P K I P E R S S P R N S E C
 E R K L E W N K S F O R R E T T A T T C E D V E N G P S P G R S P L D P O A S S S A G L V L H A T E P G H S C
 R R E S T F Y R S D S D K D L S R K A M S R N S S E E S E Q H G D D E I V T F F A O V L A S L R S V R N N I T I L
 E N L H G T S N K R S P A A S O P P V S R V N P O E S S A K T A M E I T T E V D I C H S
 I S S T A R R E I R R E I T A S E M C S N O V O Y S R A T L I K O N D V E I S
 A I M I O S C W K I N H S S I N N I S T A R C O U N T E I D I D A N E L I D N K M S I N T I N
 T I T I S I N A L E O T N D I C R O T I E S D I L I C M T H E E R R I C L O Z I N S I T A
 I S N E A E C O P A I D A T I D I L L A A M I A A S I T B A I T P C W S N E I N N I S
 S A M I T I T A Y C K L O E P O L D E M I A T A S T I R S A C T E D I V A D E K I L L I S
 E T I M E I T A S S O W I T I N D I D E V O W I T A I C O
 E R K L E W N K S F O R R E T T A T T C E D V E N G P S P G R S P L D P O A S S S A G L V L H A T E P G H S C
 R R E S T F Y R S D S D K D L S R K A M S R N S S E E S E Q H G D D E I V T F F A O V L A S L R S V R N N I T I L
 E N L H G T S N K R S P A A S O P P V S R V N P O E S S A K T A M E I T T E V D I C H S

Figure 5

PDE4B2 (acc. NM_002600) Nucleic acid sequence

```
1  gaattcctcc tctcttcacc ccgttagctg ttttcaatgt aatgctgccg tccttctctt
61  gcactgcctt ctgctgtaac acctccattc ctgtttataa ccgtgtattt attacttaat
121 gtatataatg taatgttttg taagtattta atttatatat ctaacattgc ctgccaatgg
181 tgggtgttaa tttgtgtaga aaactctgcc taagagttac gactttttct tgtaatgttt
241 tgtatttgtt attatataac ccaaactgca cttagtagag acatatggcc cccttggcag
301 agaggacagg ggtgggcttt tgttcaaagg gtctgccctt tccctgcctg agttgctact
361 tctgcacaac ccctttatga accagttttc accogaattt tgactgtttc atttagaaga
421 aaagcaaaat gagaaaaagc tttcctcatt tctccttgag atggcaaaag actcagaaat
481 gacatcacat accctaaaga accctgggat gactaaggca gagagagtct gagaaaaactc
541 tttggtgctt ctgccttttag ttttaggaca catttatgca gatgagctta taagagaccg
601 ttccctccgc cttcttcctc agaggaagtt tcttggtaga tcaccgacac ctcatccagg
661 cgggggggtt gggggaaact tggcaccagc catcccaggc agagcaccac tgtgatttgt
721 tctcctggtg gagagagctg gaaggaagga gccagcgtgc aaataatgaa ggagcacggg
781 ggcaccttca gtagaccggg aatcagcggg ggtagcgggt actctgctat ggacagcctg
841 cagccgctcc agcctaacta catgcctgtg tgtttgtttg cagaagaatc ttatcaaaaa
901 ttagcaatgg aaacgctgga ggaattagac tgggtgtttg accagctaga gaccatacag
961 acctaccggt ctgtcagtga gatggcttct aacaagttca aaagaatgct gaaccgggag
1021 ctgacacacc tctcagagat gagccgatca ggggaaccagg tgtctgaata catttcaaat
1081 actttcttag acaagcagaa tgatgtggag atcccatctc ctaccagaa agacagggag
1141 aaaaagaaaa agcagcagct catgaccagc ataagtggag tgaagaaatt aatgcatagt
1201 tcaagcctaa acaatacaag catctcacgc tttggagtca aactgaaaa tgaagatcac
1261 ctggccaagg agctggaaga cctgaacaaa tggggtctta acatctttaa tgtggctgga
1321 tattctcaca atagaccctt aacatgcatc atgtatgcta tattccagga aagagacctc
1381 ctaaagacat tcagaatctc atctgacaca tttataacct acatgatgac tttagaagac
1441 cattaccatt ctgacgtggc atatcacaac agcctgcacg ctgctgatgt agcccagctg
1501 acccatgttc tctttctac accagcatta gacgtgtctt tcacagattt ggagatcctg
1561 gctgccattt ttgcagctgc catccatgac gttgatcatc ctggagtctc caatcagttt
1621 ctcatcaaca caaattcaga acttgctttg atgtataatg atgaatctgt gttggaaaat
1681 catcaacctt ctgtgggttt caaactgctg caagaagaac actgtgacat cttcatgaat
1741 ctcaccaaga agcagcgtca gacactcagg aagatgggta ttgacatggt gttagcaact
1801 gatattgtct aacatatgag cctgctggca gacctgaaga caatggtaga aacgaagaaa
1861 gttacaagtt caggcgttct tctcctagac aactataccg atcgcattca ggtccttcgc
1921 aacatggtac actgtgcaga cctgagcaac cccaccaagt ccttggaaat gtatcggcaa
1981 tggacagacc gcatcatgga ggaatttttc cagcagggag acaaagagcg ggagagggga
2041 atggaaatta gcccaatgtg tgataaacac acagcttctg tggaaaaatc ccagggttgg
2101 ttcacgcact acattgtcca tccattgtgg gagacatggg cagatttggt acagcctgat
2161 gctcaggaca ttctcgatac cttagaagat aacaggaact ggtatcagag catgatacct
2221 caaagtccct caccaccact ggacgagcag aacagggact gccagggtct gatggagaag
2281 tttcagtttg aactgactct cgatgaggaa gattctgaag gacctgagaa ggagggagag
2341 ggacacagct atttcagcag cacaaagacg ctttgtgtga ttgatccaga aaacagagat
2401 tccctgggag agactgacat agacattgca acagaagaca agtccccctg ggatacataa
2461 tccccctctc cctgtggaga tgaacattct atccttgatg agcatgccag ctatgttgga
2521 gggccagccc accatggggg ccaagacctg cacaggacaa gggccacctg gcctttcagt
2581 tacttgagtt tggagtcaga aagcaagacc aggaagcaaa tagcagctca ggaatccca
2641 cggttgactt gccttgatgg caagcttggg ggagaggggt gaagctgttg ctgggggccg
2701 attctgatca agacacatgg cttgaaaaatg gaagacacaa aactgagaga tcattctgca
2761 ctaagtttgc ggaacttatc cccgacagtg actgaactca ctgactaata acttcattta
2821 tgaatcttct cacttgctcc tttgtctgcc aacctgtgtg ccttttttgt aaaacatttt
2881 catgtcttta aaatgcctgt tgaatacctg gagtttagta tcaacttcta cacagataag
2941 ctttcaaagt tgacaaactt ttttgactct ttctggaaaa gggaaagaaa atagtcttcc
3001 ttctttcttg ggcaatatcc ttcactttac tacagttact tttgcaaaaa gacagaaagg
3061 atacacttct aaccacattt tacttccttc cctgtttgtc cagtccaact ccacagtcac
3121 tcttaaaact tctctctgtt tgctgcctc caacagtact ttaactttt tgcgtgtaac
3181 agaataaaat tgaacaaatt agggggtaga aaggagcagt ggtgtcgttc accgtgagag
3241 tctgcataga actcagcagt gtgccctgct gtgtcttggg ccctgcccc cagaggagtt
3301 gctacagtc cgtccttctc ttgcactgcc tcttgcgcta acacctccat tcctgtttat
3361 gtaatgctgc cgtccttctc ttgcactgcc tcttgcgcta acacctccat tcctgtttat
3421 aaccgtgtat ttattactta atgtatataa tgtaatgttt tgtaagttaa taatttatat
3481 atctaacatt gcctgccaat ggtggtgtta aatttgtgta gaaaactctg cctaagagtt
3541 acgacttttt cttgtaatgt tttgtattgt gtattatata acccaaacgt cacttagtag
3601 agacatatgg ccccttggc agagaggaca ggggtgggct tttgttcaa gggctgcgc
3661 tttccctgcc tgagttgcta cttctgcaca accctttat gaaccagttt tggaaacaat
```

3721 attctcacat tagatactaa atggtttata ctgagtcttt tacttttgta tagcttgata
3781 ggggcagggg caatgggatg tagtttttac ccaggttcta tccaaatcta tgtgggcatg
3841 agttgggtta taactggatc ctactatcat tgtggctttg gttcaaaagg aaacactaca
3901 ttgctcaca gatgattctt ctgattcttc tgaatgctcc cgaactactg actttgaaga
3961 ggtagcctcc tgcctgccat taagcaggaa tgtcatgttc cagttcatta caaaagaaaa
4021 caataaaaca atgtgaattt ttataataaa aaaaaaaaaa aggaattc

Figure 6

PDE4B2 Protein sequence

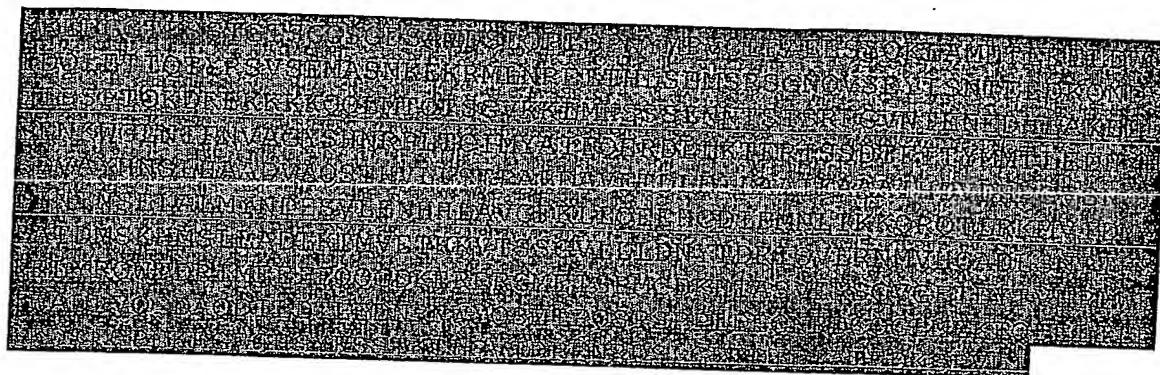
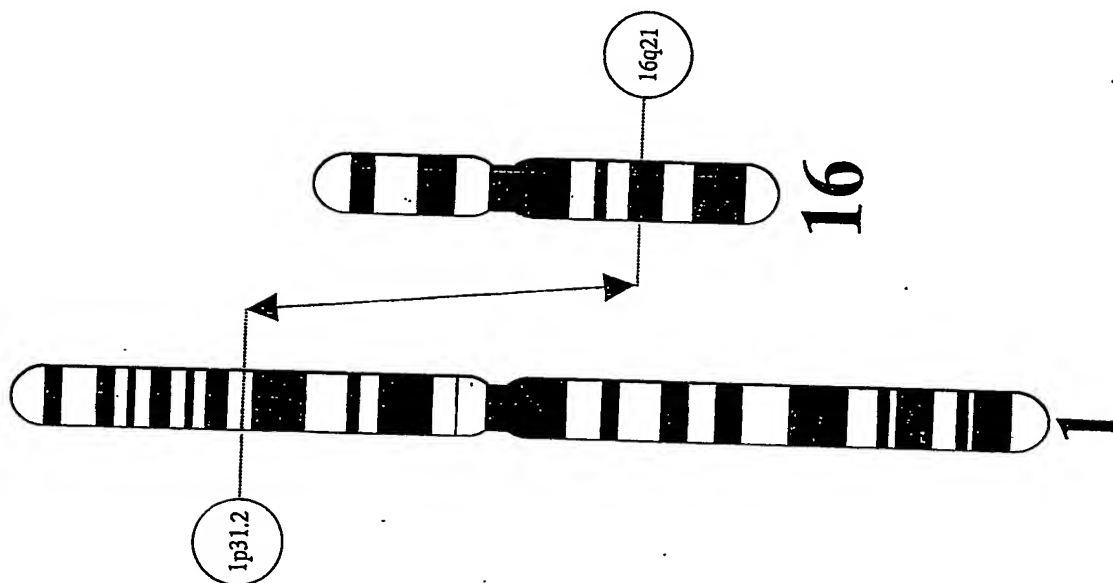


Figure 7

a)



b)

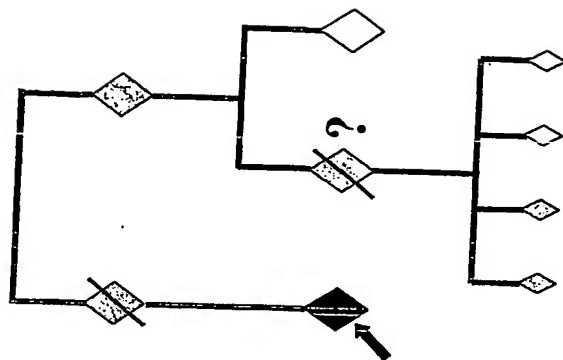


Figure 8 PDE4B gene structure (long forms)

